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Intra-articular anaesthesia mitigates established pain in experimental osteoarthritis: a preliminary study of gait impulse redistribution as a biomarker of analgesia pharmacodynamics

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SUMMARY

Objective: Develop a sensitive, functional biomarker of persistent joint pain in a large animal model of experimental osteoarthritis. Evaluate Impulse Ratio as a measure of weight distribution among supporting limbs throughout the early natural history of osteoarthritis and with local anaesthesia and analgesia.
Design: The distribution of weight bearing in the trot of 11 skeletally-mature dogs was analyzed before and after unilateral surgical intervention (cranial cruciate transection or distal femoral focal impact). The short-term effects of two analgesic treatments (intra-articular lidocaine and intra-dermal meloxicam) were then evaluated as an index of pain relief based on the redistribution of weight-bearing impulse between normal and injured limbs.
Results: Impulse Ratio was able to resolve weight redistribution between limbs in both long-term (weekly for over 400 days) and short-term (15 min intervals) joint evaluations. Joint pain relief from lidocaine administration could be reliably tracked over its brief acting time course. Meloxicam administration resulted in ambiguous results, where average weight bearing in the injured limb did not increase, but the variability of limb use changed transiently and reversibly.
Conclusion: Joint function and the role of persistent joint pain in the development of osteoarthritis can be investigated effectively and efficiently in a large animal model through the use of Impulse Ratio. Impulse Ratio can be a functionally relevant and sensitive biomarker of locomotion-related joint pain.

Introduction

The features commonly measured in osteoarthritis (OA) research include joint space narrowing, cartilage fibrillation, and proteoglycan depletion (e.g.,[1]), yet patients with OA complain primarily of joint pain. Whereas humans can communicate their joint discomfort verbally, veterinary practitioners must deduce joint discomfort in animals based on observable behaviours. In severe joint disease, animal lameness is easily detected, however, subtle changes in locomotion behaviour are difficult to detect by even highly experienced veterinarians[2]. Indeed, the subtleties of joint dysfunction in animal models of early OA are especially challenging to evaluate when lameness is used as a surrogate for chronic joint pain and the effectiveness of analgesia.

As joint pain can affect locomotion, sensitive measures of gait 'performance' could be used to quantify the effects of pain on locomotion—a functionally relevant index of OA disease progression and efficacy of treatment. However, many standard gait analysis techniques, such as three-dimensional kinematic analysis using an array of video cameras, can be complex to operate and interpret[3,4]. Indeed, kinematics are troublesome to interpret due to the high degree of variability inherent in the data set[5]. Variability—even for normal animals—can come from many sources, including actual variance between strides, motion artifacts caused by skin surface markers shifting over skeletal landmarks, limited temporal and spatial resolution of imaging technologies, and deviations from simplifying assumptions (e.g., inappropriate use of smoothing functions).

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Kinematic (motion) analyses can be valuable, but in the context of evaluating joint pain it is important that the analysis is firmly anchored in the physics and physiology that determine the motions observed. As the vast majority of limb motion in the gait cycle occurs during the 'swing phase' of the stride (when the foot is off the ground), kinematic analyses provide the highest quality information during this interval. However, during the swing phase, joint loading and presumably joint pain, are likely minimal. Conversely, joint loading is maximal during the 'stance' portion of the stride when limbs must bear: (1) the weight of the animal, (2) the impact of the limb striking the ground, and (3) the forces generated by muscles acting to move and stabilizing joints (which can be many times body weight). If joint pain is related to the force transmitted through a joint, and the proportion of body weight supported by a given limb determines its joint loading, then the *redistribution of body weight support among limbs during stance* would likely be a sensitive metric of joint pain, and possibly serve as a functional biomarker of pain related to locomotion. Moreover, reducing or alleviating joint pain (e.g., through analgesia or local anaesthesia) would be expected to restore limb weight bearing during stance towards a normal (symmetrical) condition.

Newly developed inertial sensors can evaluate trunk motion (representing centre-of-mass)[6] or body components particularly responsive to lameness (e.g., head motion in horses)[7] and have substantial potential for distinguishing subtle changes in limb use. This technology measures the effects of differences in limb loading, yet does not measure limb loading directly. In this study, we use independent measurements of the front and hindlimb ground-reaction forces to provide a dynamic analysis of the whole-body distribution of weight bearing in the canine trot. Redistribution of weight bearing among limbs, as indicated by the proportion of vertical impulse provided by each limb over the course of a complete stride (where a stride cycle is the fundamental unit of gait), is then compared in animals with two types of experimental OA and with two types of analgesia. By focussing on the support role of each limb during the stance phase it is possible to detect relatively subtle changes in weight-support redistribution among limbs. Hence, we chose to evaluate dynamic weight redistribution during the natural history of post-traumatic joint injury as a biomarker of chronic joint pain. The spatial and temporal resolution of this analysis, and its efficiency, enabled the rapid measurement of changes in joint function over the effective course of a relatively fast-acting analgesic. This allowed its use as an index of pain relief in these models of experimental OA.

Materials and methods

Animals

Eleven skeletally-mature, mixed-breed dogs of both sexes were used with institutional ethics approval according to the guidelines of the Canadian Council on Animal Care. Animals ranged in age from 2 to 6 years, ranged in body mass from 17.5 to 32.0 kg [mean ± standard deviation (SD); 25.5 ± 4.7].

Individual limb assessment from whole-body ground-reaction force—Impulse Ratio

Whole-body ground-reaction force analysis[8,9] was used to determine the proportion of body weight supported by each limb of trotting dogs (the canine trot is most commonly characterized by alternating footfalls of diagonal hindlimb–forelimb pairs). The animal must provide a total ground-reaction force equivalent to its body weight to support itself over a complete stride cycle, regardless of its speed. This physical requirement was used to determine how the required load is shared among the available limbs (ipsilateral and contralateral, forelimb and hindlimbs). The weight bearing contributed by each limb was quantified as the *Impulse Ratio*—the proportion of total vertical impulse that is contributed by each individual limb over a complete stride.

Analyzing a complete stride requires a serial force-plate array[10] so that front and rear ground-reaction forces can be measured simultaneously and independently. A linear array of four force plates was used, with each plate 0.61 × 0.30 m (2.45 m total length with 0.005 m between platforms to prevent interference). These were placed in the centre of a runway 7.30 × 0.30 m, raised to meet the height of the force platforms (0.10 m). The force platforms were custom-built to match the stride length of the study animals (so that force recordings could be acquired from independent limb contacts when each diagonal limb pair made simultaneous contact) based on designs in the literature[11,12] modified for canine gait[8].

Vertical ground-reaction force (and apparent applied impulse) is sensitive to small horizontal accelerations (positive or negative[9,13]). The Impulse Ratio analysis of Lee *et al.*[9] accounted for these accelerations by calculating the torque applied by the horizontal component of the force to the centre-of-mass of the animal over the stride cycle and normalized the impulse distribution to steady-state (constant speed[8,10]). This provides a substantial increase in analysis precision[9] and facilitates the characterization of gait because acceleration over the force-platform array need not be controlled or restricted (indeed, runs involving horizontal acceleration actually improve the precision of impulse distribution characterization for steady-state locomotion[9]).

Output of the force plate sensors was amplified (SCXI 1121 strain conditioning modules operating in an SCXI 1000 amplifier), and digitized (NI USB-6251 multifunction correlated digital I/O) to a laptop computer using a custom acquisition program (written in LabView v8.5, National Instruments). These force records were analyzed using a custom application (written in Matlab, after[9]), which calculates the independent vertical and horizontal impulse for each limb within each stride. The individual limb impulses (accounting for fore-aft torque effects) were combined to determine whole-body impulse over the stride, and the *vertical impulse distribution* was assessed as the Impulse Ratio contributed by each limb to support the animal's body weight. Video recordings of the animals trotting across the force platforms enabled the identification of which limbs were responsible for the specific ground-reaction forces and impulses.

Limb function (as indicated by the Impulse Ratio) was evaluated prior to and at various intervals following surgery. Typically 10–16 individual strides were collected as each animal trotted across the force-plate array at each time point (to constitute one trial). To evaluate the contribution of pain to the gait performance of animals with experimental OA, repeated trials on each animal were performed before and after anaesthesia or analgesia, and before and after joint injury surgery.

Surgical models of OA

Two surgical models were used to mimic OA: unilateral cruciate ligament transection ($n = 7$) and unilateral focal-impact injury ($N = 4$ animals). Transection of the cruciate ligament was performed through a lateral para-patellar incision (after[14]). Briefly, one of the stifle joints was chosen in alternating fashion, and prepared for surgery. After joint capsule incision, the cranial cruciate ligament was elevated and cleanly divided with a #11 scalpel. The contralateral stifle served as a non-operated, internal control. Unilateral focal-impact injury was performed through a medial para-patellar incision cranial to the medial femoral condyle (after[15]). Briefly, the stifle was held in flexion, the patellar retinaculum and joint capsule were

incised and held with a Gelpi retractor. A spring-loaded, custom-fabricated impactor was attached to a 4-mm-diameter, flat-ended, stainless steel tip and placed in full contact with the medial femoral condyle. A calibrated impact of 28 MPa was imparted to the articular surface, a load that disrupts cartilage, causes some chondrocyte death, and leads slowly to OA[15]. The contralateral stifle served as a non-operated control. All animals received a fentanyl patch (75 μg) for 3 days of post-operative analgesia.

Joint fluid sampling and intra-articular anaesthesia

Arthrocentesis was performed prior to injecting intra-articular lidocaine HCl (1% solution, neat). Joint fluid was drawn into a syringe ensuring that the needle was in the joint space, the syringe was removed and changed to a syringe containing 0.5 mL lidocaine, which was injected. This dose of lidocaine is comparable to that for intra-articular use in humans (e.g.,[16]) and low enough to avoid cartilage cytotoxicity (e.g.,[17]).

Meloxicam analgesia

Meloxicam, the clinical standard for joint pain relief in veterinary medicine, was used as a comparator with intra-articular analgesia. Five animals were studied at various stages of cruciate insufficiency or femoral contusion. Briefly, all animals received a single dose of meloxicam intra-dermally (0.2 mg/kg), which results in peak plasma concentrations at 2.5 h[18], and peak joint fluid concentrations at approximately 3 h[19]. Gait performance was evaluated for all animals just prior to meloxicam injection, and at 2.5 and 27.5 h afterwards.

Termination of experiments

Animals were assessed periodically by gait analysis and euthanized after various intervals (3–17 months after surgery, depending on the design of other ongoing experiments). Macroscopic assessments of articular cartilage were graded using a modification of the Outerbridge scoring system for canine cartilage[20].

Statistical analysis

The curvilinear changes in Impulse Ratio over an extended period for two dogs (A and B) were characterized by fitting the post-surgical values with both a second-order polynomial and a power function. This analysis was performed using a commercial graphing and analysis program (Kaleidagraph v3.2, Synergy Software, Reading, PA). Reliability of fit is reported using the coefficient of determination (R^2).

The Impulse Ratio changes resulting from intra-articular lidocaine injection were tested against the null hypothesis – no effect, as indicated by a linear relationship with zero slope. As it was anticipated that any effect on impulse distribution would be subtle and transient, Impulse Ratio and time values were fit with the polynomial $y = a + bx + cx^2$ where y = Impulse Ratio and x = time in minutes. The probability that the coefficients are non-zero due to chance alone was tested for the quadratic (transient effects vs null hypothesis of no effect). Results are reported as probability (P) values. These analyses were performed in R (version 2.14.1, R Foundation for Statistical Computing, Vienna, Austria – for further explanation of the analysis see Ref.[21]).

Results

Animals

Animals were readily trained to trot across the force-platform array. The typical time for collecting 10–16 strides (one trial) was less than 5 min. Surgery and recovery were uneventful, save for one animal (with a cruciate transection) with a visible limp that persisted for more than 3 weeks. Joint fluid smears were negative for

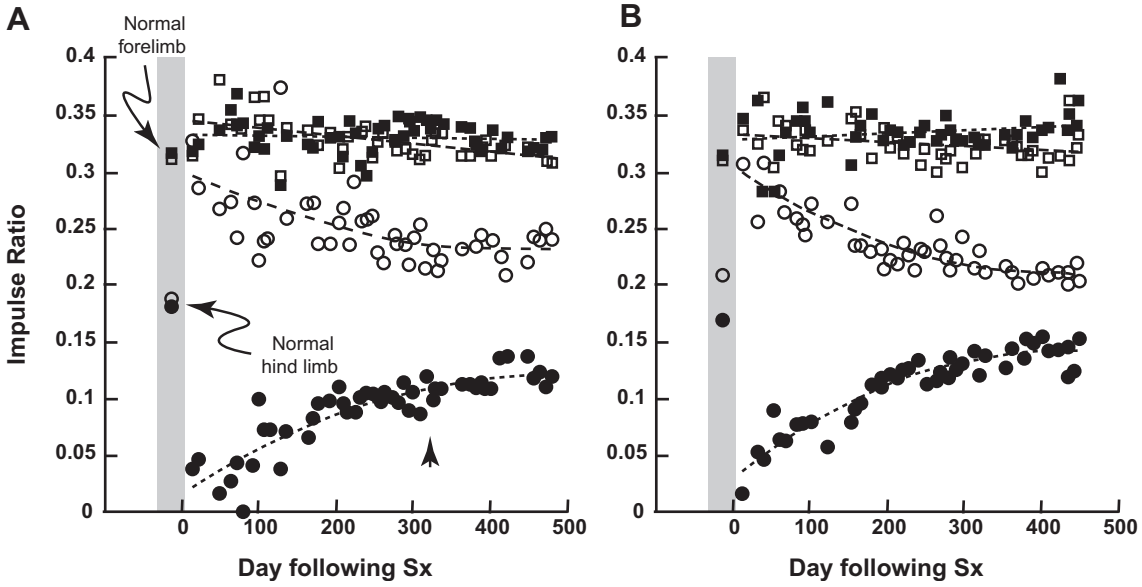

Fig. 1. Impulse Ratio prior to, and following recovery from unilateral cruciate transection in two dogs followed for an extended period. "Diagonal pairs" of hindlimbs and forelimbs alternately carry the entirety of body mass during reciprocating trotting gait. Hence, the ipsilateral (cruciate-transected) hindlimb is indicated as solid circles and the contralateral-side forelimb is indicated as solid squares (i.e., solid figures represent a diagonal pair). The opposite diagonal pair of limbs (the contralateral, non-operated hindlimb and ipsilateral forelimb) are indicated by unfilled circles and squares, respectively. The earliest set of points (day −1) is the pre-operative condition (highlighted by grey shading). Note that following surgery a decrease in impulse by the ipsilateral (injured) hindlimb induces compensations in the other three limbs (primarily the contralateral hindlimb) so that an impulse equivalent to the animal's entire weight is provided over the complete stride (i.e., the summed Impulse Ratio of all limbs *must* equal 1.0 – indicating an average vertical force equal to body weight is applied over the period of the stride). Each point on the graph represents the mean of 6–12 full strides. The vertical arrow in A indicates the point in the post-operative recovery when the data shown in Fig. 2 were taken (day 325 of post-operative recovery). A: dog A, a 27.5 kg mixed breed. B: dog B, a 32.0 kg mixed breed.

acute inflammation or infection. At necropsy, all animals had macroscopic and microscopic changes in their injured joints consistent with early post-traumatic OA at the time of necropsy (data to be reported elsewhere). Outerbridge scores never exceeded stage 2 for cruciate-transected joints, but reached stage 3 just at the impact site of two of four impact-injured joints.

Impulse redistribution following long-term cruciate transection

The impulse distribution was measured prior to, and for nearly 500 days of recovery after, cruciate ligament transection in two dogs. Both had similar impulse redistribution trajectories (Fig. 1, Table I). Prior to surgery, a normal impulse distribution is observed, with each of the two forelimbs carrying approximately 0.325 (combined 0.65) of the vertical impulse, while each of the two hindlimbs carried approximately 0.175 (combined 0.35; Fig. 1). This compares to Impulse Ratio measures reported previously from Labrador retrievers (combined forelimbs 0.69, hindlimbs 0.31) and Greyhounds (combined forelimbs 0.74, hindlimbs 0.26[9]).

Immediately following surgery the Impulse Ratio contributed by the cruciate-transected hindlimb fell to below 0.05 (71% decrease from pre-operative value) while the contribution from its contralateral, non-operated hindlimb nearly doubled (increasing to approximately 0.30). Small increases in the vertical impulse carried by the forelimbs were also observed. This dramatic redistribution of hindlimb weight bearing was followed by a gradual increase over an extended period in the impulse contribution provided by the cruciate-transected limb. This increase in Impulse Ratio reached an asymptote at 0.12 (31% decrease from pre-operative value) after approximately 420 days of recovery.

The Impulse Ratio carried by the forelimbs changed to a much lesser degree than the hindlimbs following surgery. In Fig. 1 these data are fit with linear regressions to indicate the trend line of each. Although subtle changes were detected in the forelimb Impulse Ratio as recovery progressed, and both animals tracked over an extended period responded similarly, the slopes identified were too flat to generate a significant regression.

The Impulse Ratio applied by each hindlimb changed over time. The recovery data were fit well by both second-order polynomial and power function regressions, with both having about equal statistical fit to the data (Table I). Figure 1(A) shows the second-order polynomial trends for dog A tracked for 478 days and Fig. 1(B) shows these trends for dog B, a 32 kg mixed breed, tracked over 448 days.

Intra-articular lidocaine administration

Following lidocaine administration, some subtle changes in impulse distribution in all limbs are typically seen for cruciate-deficient animals (this was observed for all of the dogs tested following 3 or more months of recovery, but one animal tested at 3, 5 and 7 weeks of recovery showed no change in impulse following intra-articular lidocaine). For example, Fig. 2 shows the response of dog A [seen in Fig. 1(A)] tested at day 325 post-operatively [vertical arrow in Fig. 1(A)]. On the day prior to lidocaine treatment substantial asymmetry of weight bearing can be seen in the hindlimbs (the cruciate-transected limb imparts ~1/3 less impulse compared to pre-operative values, while the contralateral hindlimb carries ~1/3 greater impulse). Shortly after lidocaine administration, the injured limb bears a *larger* portion of the impulse required to support the animal's body weight, while the Impulse Ratio *falls* in the contralateral hindlimb. Corresponding changes in impulse distribution can be seen in the forelimbs, indicating that their

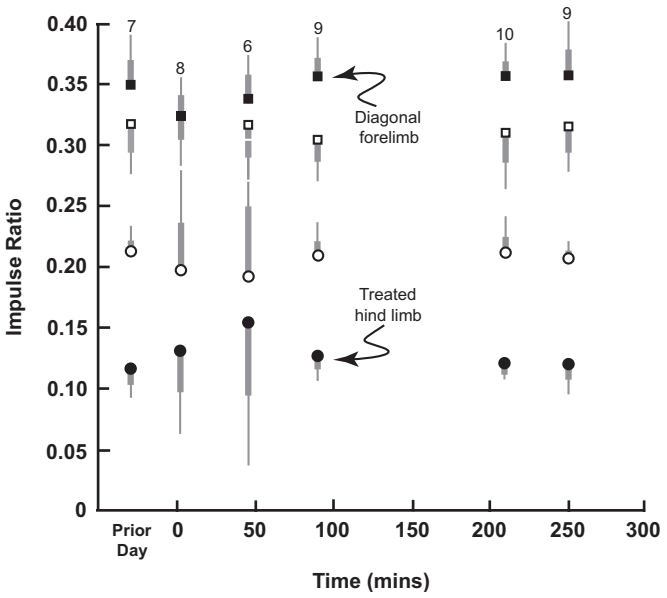

Fig. 2. Impulse redistribution in dog A as a result of intra-articular administration of 0.5 mL lidocaine at time 0 [administered at day 325 of cruciate transectomy recovery; see vertical arrow Fig. 1(A)]. During the period of expected analgesic effect, the treated limb increases its proportion of weight bearing, while that of the contralateral limb decreases. This 'redistribution' of weight bearing extends to the forelimbs as well, where the increased load bearing of the treated hindlimb is matched by transient decreases in the load carried by its diagonal forelimb partner. As the analgesia dissipates, weight bearing in each limb is maintained at levels consistent with those observed prior to analgesic administration. Symbols as in Fig. 1. Grey rectangles extending from symbols indicate 1 standard deviation of the mean, grey lines extending from symbols indicate 95% confidence intervals (variability is symmetrical and for clarity is plotted only in one direction for each time point). Numbers over the plotted data points indicate the number of valid strides available for analysis. See Table II for an evaluation of trends described.

Table I
Polynomial and power function regression Impulse Ratio coefficients for long-term recovery for two cruciate-transected dogs

Dog	Limb	#Days	a	b	c	R^2
Second-order polynomial regression: Impulse Ratio vs day following surgery where Impulse Ratio $= a + b\,(day) + c\,(day)^2$						
A	Transected	478	0.017	4.4×10^{-4}	-4.70×10^{-7}	0.778
	Contralateral		0.300	-3.2×10^{-4}	3.80×10^{-7}	0.436
B	Transected	448	0.031	5.0×10^{-4}	-6.40×10^{-7}	0.882
	Contralateral		0.302	-4.0×10^{-4}	5.37×10^{-7}	0.747
Dog	Limb	#Days	a	b		R^2
Power function regression: Impulse Ratio vs day following surgery where Impulse Ratio $= a\,(day)^b$						
A	Transected	478	6.7×10^{-3}	0.481		0.785
	Contralateral		0.378	−0.080		0.457
B	Transected	448	8.2×10^{-3}	0.483		0.863
	Contralateral		0.451	−0.126		0.723

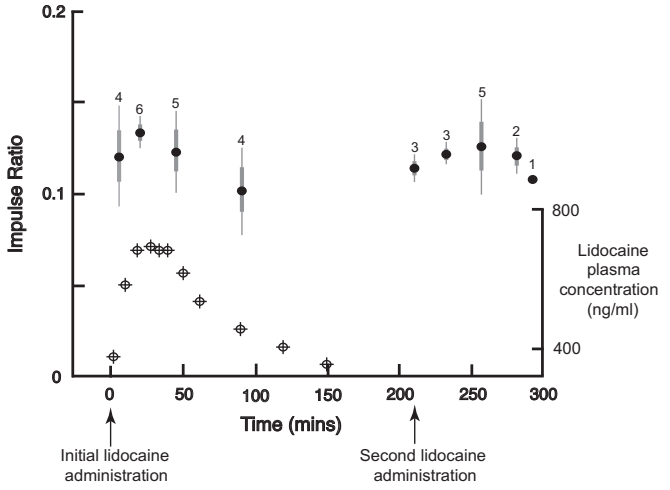

Fig. 3. A detailed evaluation of Impulse Ratio changes (solid circles) in the cruciate-transected hindlimb of dog C (21.5 kg mixed breed, 300 days following cruciate transection). A transient increase and then decrease in weight bearing is seen over a 90-min period (see statistically verified trend, Table II). A second dose of intra-articular lidocaine was administered 220 min following the first dose, where a similar transient change (statistically verified in Table II). Grey rectangles extending from symbols indicate 1 standard deviation of the mean, grey lines extending from symbols indicate 95% confidence intervals, other annotations as in Fig. 2. The weight-bearing response is compared to the time course of plasma concentration changes for a lidocaine/epinephrine injection in humans (circled crosses, as reported by Ref.[22]).

contribution to weight bearing is affected by the increased contribution by the cruciate-transected limb. The lidocaine treatment effect peaks 45 min after intra-articular administration and diminishes thereafter; by 90 min the impulse distribution in all limbs returns to pre-administration levels.

In Fig. 3 the Impulse Ratio changes for the injured limb of dog C (21.5 kg, 300 days following cruciate transection) are analyzed for two sequential intra-articular injections of lidocaine. The impulse carried by the cruciate-transected limb changes after lidocaine injection in a measureable manner, where the impulse carried by this limb *increases* shortly after injection, then falls to pre-administration levels at 90 min. This first transient change in Impulse Ratio is significant (Table II) and so is the transient change observed after a second intra-articular injection 220 min after the initial dose (after the first dose had dissipated). In Fig. 3 these changes are compared to the time course of plasma lidocaine concentration in humans (data from Ref.[22]). Corresponding *decreases* in impulse occurred in the contralateral hindlimb (data not shown) demonstrating that the impulse contribution of the ipsilateral and contralateral limbs are linked.

For animals with no appreciable gait abnormalities, lidocaine administration evokes no measureable changes in impulse distribution among limbs. Figure 4 shows the Impulse Ratios before and following lidocaine administration in a normal animal and in an animal with a stable joint that had sustained a focal-impact injury (69 days after surgery). All four of the focal-impact animals showed no appreciable limp clinically or by gait analysis (impact initiates a slow articular cartilage breakdown without destabilizing the joint) and this was also the case for all seven normal (pre-cruciate surgery) animals tested. In both circumstances, no detectable change in Impulse Ratio is seen after intra-articular lidocaine administration.

Intra-dermal meloxicam administration

The impulse distribution following intra-dermal meloxicam administration differed substantially from that following intra-articular lidocaine. As expected (and as seen with lidocaine as well), there was no impulse redistribution in normal animals (data not shown). This was also the case for some of the animals treated with focal impact. Nevertheless, in three of the four focal-impact animals there was a substantial increase in Impulse Ratio *variance* in the impacted limb, *which was not apparent in the contralateral limb*, 2.5 h following administration, when the analgesic effect of meloxicam peaks (Fig. 5). The following day *this variance had disappeared* and the impulse distribution was comparable to pre-administration levels. This was also seen in the cruciate-transected animals that received meloxicam, where clear increases in variance of impulse for the injured limb were observed, but *not* in the contralateral hindlimb.

Discussion

In humans and animals, decreased mobility due to pain is a defining feature throughout the natural history of OA. Hence, alterations in gait and joint loading patterns have long been targeted by researchers as a means to noninvasively monitor the pathogenesis of OA (particularly in animal models) and as an index of chronic joint pain[23,24]. The present studies, though preliminary in nature due to the limited number of animals investigated and range of conditions analyzed, support the assertion of Bertram *et al.*[8] and Kennedy *et al.*[10] that the Impulse Ratio can be used as a relatively sensitive and time-effective method of monitoring the relative load support of each limb to carry the body mass of trotting quadrupeds. Impulse Ratio is a measure of gait directly related to the functional capabilities of the animal and differs substantially from other kinetic analyses that utilize ground-reaction force derived from less than a full stride cycle. Although other force and impulse-based analyses can detect some changes[25], they do not easily track subtle redistributions of weight or account for the complete weight support strategy the animal uses (increases in the contralateral limb loading do not match affected limb decreases). Through the use of an array of four force platforms and an analysis based on the full stride cycle, it is possible to monitor the distribution of applied impulse among all limbs that

Table II
Evaluation of data trends for intra-articular lidocaine treatment. Dog A: analysis of all limbs for a single treatment (data shown in Fig. 2). Dog C: analysis of the affected limb for sequential administrations 220 min apart (second treatment after the effects of the first washed out of the system). The data were fit with the relation $y = a + bx + cx^2$ where y = Impulse Ratio of limb and x = time in minutes after lidocaine administration. *P*-values indicate that probability that the coefficients are non-zero due to chance alone, that is, that the transient change in impulse value differs significantly from no effect. All statistical analyses were performed in R (Version 2.14.1, R Foundation for Statistical Computing, Vienna, Austria)

Dog	limb	*a*	*P*-value	*b*	*P*-value	*c*	*P*-value
A	Left hind (transected)	0.1375	<0.0001	5.516e-4	0.0665	−7.143e-6	0.0860
	Right hind (contralateral non-operated)	0.1966	<0.0001	−3.848e-04	0.265	5.921e-06	0.219
	Left front (diagonal to non-operated)	0.3223	<0.0001	2.716e-05	0.872	−2.486e-06	0.295
	Right front (diagonal to transected)	0.3318	<0.0001	−3.156e-04	0.1005	6.788e-06	0.0138
C	Right hind (transected) initial lidocaine administration	0.1246	<0.0001	3.464e-4	0.2410	−6.726e-6	0.0381
	Right hind (transected) follow-up lidocaine administration	0.1216	<0.0001	2.735e-4	0.0951	−5.264e-6	0.0337

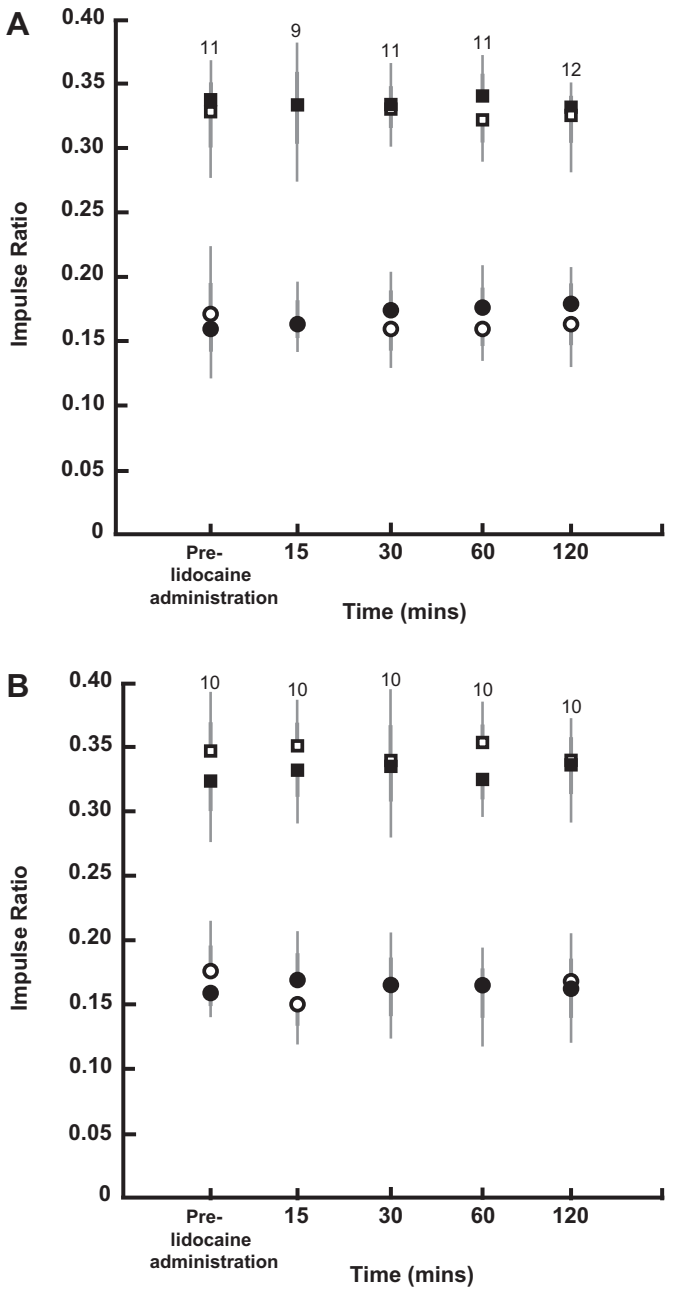

Fig. 4. Whole-body Impulse Ratios for two dogs with no clinical signs of gait abnormality followed after intra-articular lidocaine administration (at time zero). Upper panel (A), dog D (18.3 kg mixed breed) with no surgical intervention, serving as a normal control; lower panel, dog E (31.1 kg mixed breed) that had been treated with distal femoral focal impact 5 weeks prior to analgesic administration. No appreciable changes in impulse distribution are seen with intra-articular analgesic, even in the animals with focal cartilage impact. Grey rectangles extending from symbols indicate 1 standard deviation of the mean, grey lines extending from symbols indicate 95% confidence intervals. Symbols and annotations as in Fig. 2.

provides body weight support. By accounting for the full body support it is possible to follow both decreases and increases in the contribution of any limb and account for all aspects of weight support during dynamic changes in locomotion.

By appropriately instrumenting the environment in which an animal freely moves (i.e., the surface on which the dog trots), it is possible to allow the animal to move as it chooses. This substantially decreases the time necessary to acquire adequate gait data because the animal's speed or acceleration need not be constrained. Likewise, it does not matter which limb hits on which platform (limb identity was determined later from lateral video recordings that were used only for this purpose). It was only necessary to have the animal make contact with three consecutive force plates with alternating diagonal limb pairs to have acceptable data to analyze. For our analysis we typically collected 10–16 strides at each time point, and this required 3–7 min of data collection. Relatively few runs were rejected (e.g., only those with 'inappropriate' contact with the force platforms such as double simultaneous contacts on the same platform – sometimes a problem with severe lameness – or accidental contact at the boundary between two platforms). Using this analytic approach makes it possible to quantify short-duration changes in individual limb weight bearing, such as the transient effects of rapid-acting local anaesthetics and analgesics, thereby directly measuring the effects of established joint pain in a quadrupedal model of OA development and progression.

Long-term monitoring of impulse redistribution following recovery from unilateral cruciate transection showed that, immediately following surgery, the expected substantial decrease in load bearing by the ipsilateral limb occurred. This was accompanied by compensatory increases in load bearing by the contralateral limb and, to a much lesser degree, increases in load bearing of the forelimbs as well. Such changes in load bearing of the uninjured limbs, required for the animal to support its full body weight with the reduced contribution from the injured limb, may be responsible for the influence of ipsilateral surgery on the contralateral limb, and add to the complications of using a contralateral limb as an internal control[26,27]. As post-operative recovery progresses, a gradual redistribution of limb weight bearing occurs consistently over an extended period, even well beyond a year. The pattern of weight redistribution is consistent between the two animals analyzed long-term in this study, and previous reports in dogs[25] and in cats[28]. Weight bearing by the injured limbs plateaued by day 400, which is still only 2/3 of their pre-operative Impulse Ratio. Likewise, the contralateral hindlimbs decreased their contribution to support as the injured limbs increased their contribution, but the two limbs did not return to their pre-operative contributions over the term of this study.

Impulse Ratio as an index of limb load redistribution allows the quantification of changes in gait performance over the time course of a fast-acting agent such as lidocaine. Even following substantial recovery (325 days), intra-articular lidocaine administration led to measureable increase in the Impulse Ratio carried by the injured limb (with concomitant decrease in the impulse applied by its forelimb diagonal partner). This indicates that at least some of the weight-bearing asymmetry that results from cruciate transection can be attributed to joint pain, even after extended recovery. Whereas it is possible that some of the lidocaine-induced alterations in Impulse Ratio could be attributed to nervous elements other than pain-sensitive endings, in particular to joint capsule proprioceptors, the lack of any appreciable lidocaine effect on the gait of normal and focal-impact animals suggests that joint proprioceptors play a minor role in the redistribution of weight bearing as measured by Impulse Ratio.

Increase in impulse bearing by the cruciate-deficient limb following intra-articular lidocaine administration appears repeatable even within hours (Fig. 3). Some of this effect can undoubtedly be attributed to the immediate reduction of joint pain by the analgesic effect, yet the current analysis may underestimate this effect. Some features of gait asymmetry may persist due to neurologic patterning even if the original stimulus for asymmetry (pain) is removed[29]. This may be particularly important for these dogs who have habituated to this manner of movement for an extended period, a concept supported by reports of central neuroplasticity after delayed deafferentation after cruciate insufficiency[30].

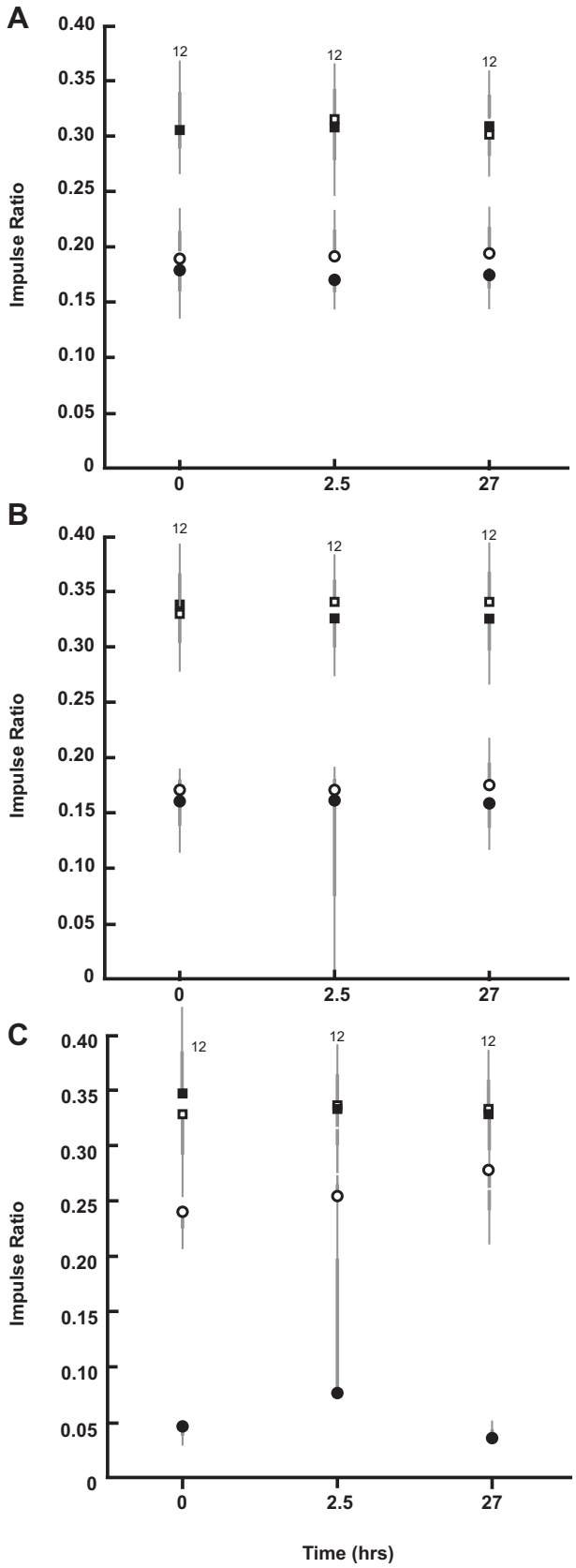

Fig. 5. The effect of intra-dermal meloxicam treatment on three dogs; (A) dog F (30.3 kg mixed breed) treated with focal impact (8 weeks prior to analysis), (B) dog G (22.0 kg mixed breed) treated with a similar focal impact (7 weeks prior to analysis), (C), dog H (29.5 kg mixed breed) with cruciate transection (13 weeks prior to analysis). Results differed among animals but the majority (four of six animals) showed a substantial increase in variability of weight bearing in the injured limb—*but not the contralateral control hindlimb*—2.5 h following administration, when the greatest physiological effect is expected. Grey rectangles extending from symbols indicate 1 standard deviation of the mean, grey lines extending from symbols indicate 95% confidence intervals. Symbols and annotations as in Fig. 2.

It is known that lidocaine is a fast-acting and rapidly-metabolized analgesic, but its specific influence on the perception of pain in the dog when administered intra-articularly is unknown. We compared the time course of the externally measured impulse redistribution in a dog treated this way, with the plasma concentration of lidocaine in humans (Fig. 3), the half-life of which is similar to that of plasma and synovial fluid in normal dogs[19]. Although not a direct indication of the activity of lidocaine within a cruciate-transected joint, the time course of changes in Impulse Ratio and lidocaine half-life are remarkably coincident, and are highly suggestive that changes in Impulse Ratio of injured joints correspond to changes in joint pain relief. Similar administration of intra-articular lidocaine to dogs with no apparent gait abnormality had no discernable effect on impulse distribution between hindlimbs (Fig. 4). This held for those animals treated with a focal impact, indicating that disabling joint pain is not a prominent characteristic of this type of injury, at least in the early stages after initial impact.

Systemic non-steroidal anti-inflammatory medications, such as the meloxicam, are commonly administered to canines with OA. In the present study, those animals treated with meloxicam showed no significant increase in average weight bearing by the injured limb. However, in some animals there was an increase in the variance of Impulse Ratio of the injured limb during each stride. This unanticipated result indicates that a single dose of meloxicam has a measureable effect on joint function, as reported previously with meloxicam treatment of acute sodium urate-induced synovitis[31], but the load bearing response of the animal is not as easily interpreted as the direct joint anaesthetic effect produced by intra-articular lidocaine. As meloxicam analgesia works through a different mechanism than lidocaine and has additional anti-inflammatory actions (that appear to be more influential in synovitis than chronic mechanical injuries), it may be that multiple or higher doses of meloxicam are needed to achieve detectable joint analgesia in these models of chronic OA. This is supported by a clinical trial wherein month-long meloxicam treatment reportedly caused subjective improvement of established canine OA[32]. In the present studies, weight bearing of the injured limb increases for some strides, as expected, but not consistently between strides. It is possible that pain is relieved, but other joint sensitivity (such as that of instability) remains. However, such a hypothesis is inconsistent with some of the focal-impact animals (Fig. 5), which do not have measureable changes in Impulse Ratio and show no response to intra-articular lidocaine. This result supports the possibility that some non-steroidal anti-inflammatory drugs could interfere with the neuromuscular control (e.g.,[33]). More thorough and longer-term studies of this effect are needed before any definitive conclusions can be drawn.

The present studies illustrate the utility of Impulse Ratio as a sensitive measure of limb use in canine locomotion and its effectiveness at enabling the timely assessment of the dynamic changes that occur as a result of both long and short-term alterations to joint function and discomfort. Hence, the Impulse Ratio is a novel and potentially sensitive tool for monitoring the influence of pain-related effects on locomotion in preclinical models of OA. Using this technique, it was possible to determine that the persistent gait asymmetry that characterizes long-term cruciate insufficiency is due in part to persistent joint pain. Likewise, an alternate method of experimentally simulating a slower joint degenerative processes,

distal femoral impact, showed no indication of persistent joint pain (at the 3-month interval studied), while a single administration of an non-steroidal anti-inflammatory drug resulted in unexpected responses from many of the animals. Impulse Ratio shows considerable promise to be a sensitive functional biomarker of chronic joint pain—and pain relief—in preclinical models of early OA, and as such, this approach has the potential to better resolve joint function in the early pathogenesis of joint diseases as well as to better define the efficacy of both surgical and pharmacological treatments of OA.

Author contributions

Drs Matyas, Hurtig, and Bertram were responsible for conceptualizing and writing this paper as well as some data collection and analyses. Dr Gutmann and Ms Randev were responsible for the collection, analysis, and interpretation of the data.

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Conflict of interests

None of the authors have any competing interests.

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